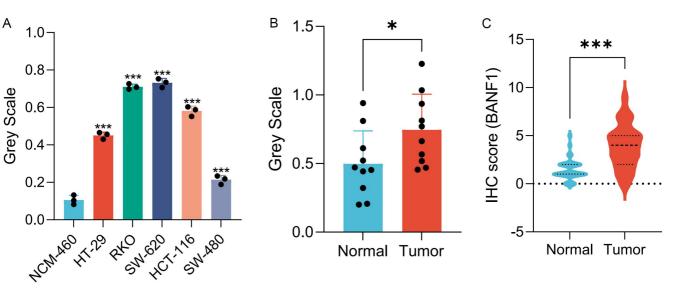
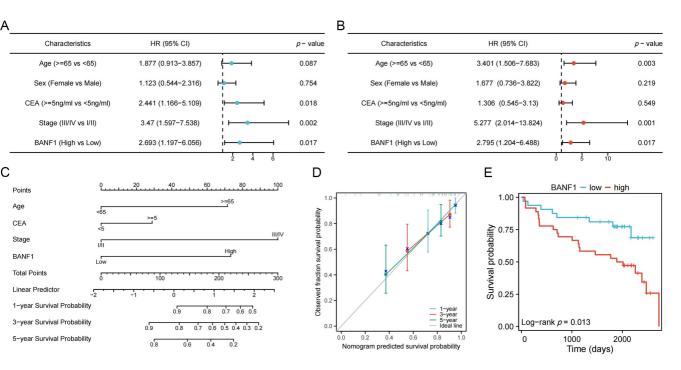


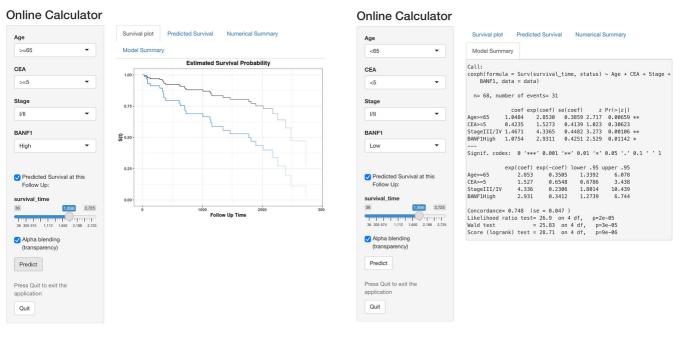
**Supplementary Figure S1:** BANF1 is extensively expressed across a range of cell types, including epithelial cells, tumor cells, and immune cells, in various malignant tumors such as invasive breast cancer (A), colorectal adenocarcinoma (B), glioma (C), clear cell renal carcinoma (D), hepatocellular carcinoma (E), non-small cell lung cancer (F), pancreatic cancer (G), and prostate cancer (H).



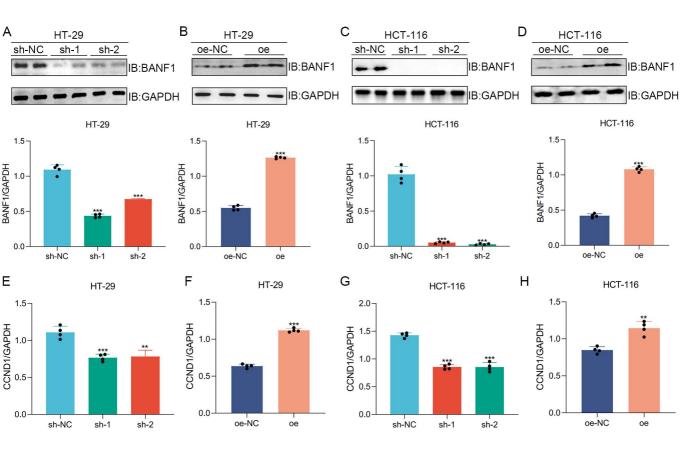
**Supplementary Figure S2:** The quantitative analysis of western blotting data for BANF1 in CRC cell lines (A) and tissues (B), along with IHC staining results from 68 pairs of CRC and adjacent normal tissues (C), is presented. \*p < 0.05; \*\*\*p < 0.001.



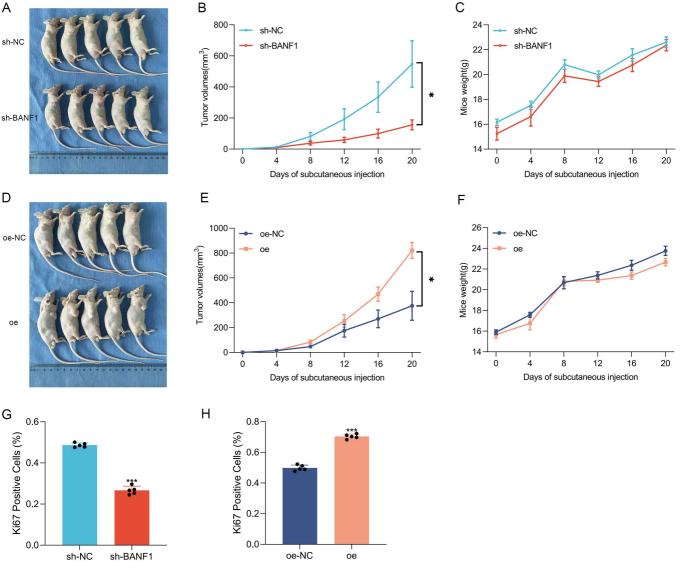
**Supplementary Figure S3:** The findings from the univariate (A) and multivariate (B) Cox regression analyses indicate that BANF1 exhibits strong predictive capabilities for overall survival. By incorporating BANF1 expression along with other key clinical variables such as age, preoperative CEA levels, and AJCC staging, a nomogram model was constructed (C). The accuracy of this nomogram was assessed through a calibration plot (D). Additionally, Kaplan-Meier survival analyses revealed that colorectal cancer (CRC) patients with low BANF1 expression had a significantly better prognosis, characterized by longer survival times, compared to those with high BANF1 expression (E).



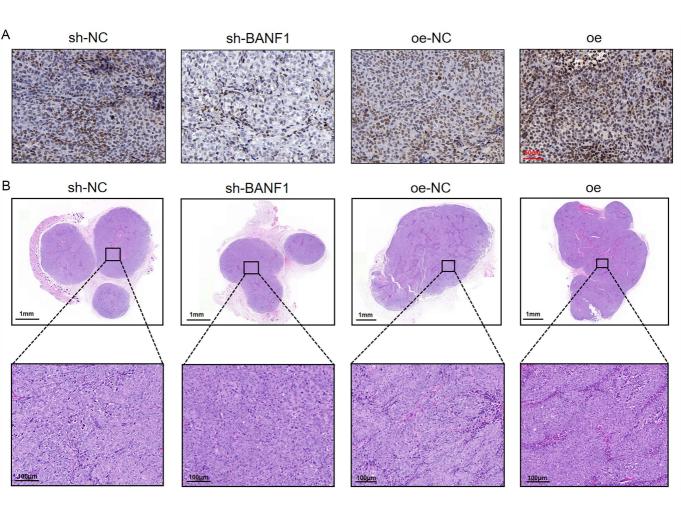
**Supplementary Figure S4:** To enhance the clinical applicability of this model, we have developed an online web-based calculator (accessible at https://doctorwang.shinyapps.io/BANF1/), designed to offer valuable insights for clinical practice and inform treatment decision-making.



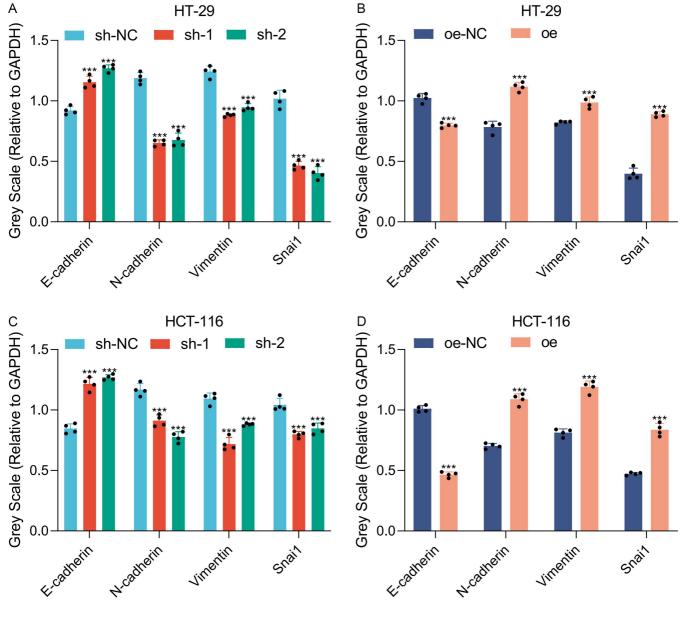
**Supplementary Figure S5:** (A-D) The efficacy of BANF1 knockdown and overexpression was further corroborated using Western blot analysis. (E-H) A quantitative analysis of the western blotting results for CCND1 was also conducted. \*p < 0.01; \*\*p < 0.001.



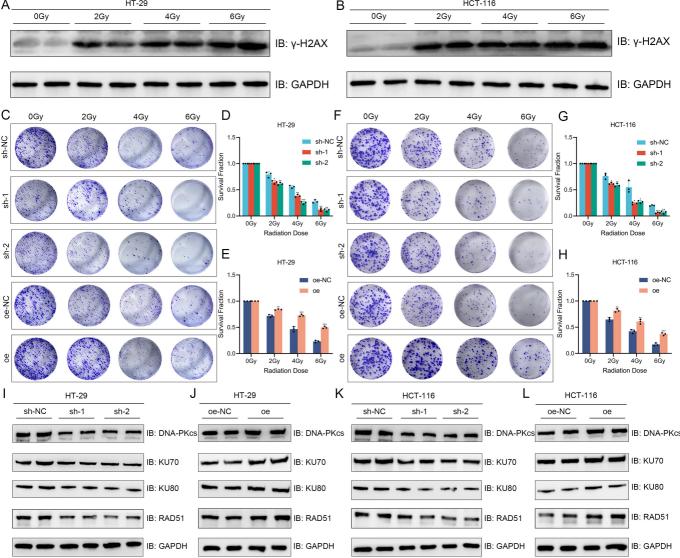
**Supplementary Figure S6:** Mice were injected with control and BANF1 knockdown cells (A), and measurements of tumor volume (B) and body weight (C) were recorded every four days. Similarly, mice injected with control and BANF1 overexpression cells (D) had their tumor volumes (E) and body weights (F) documented. The tumors from the BANF1 knockdown group exhibited lower Ki67 staining intensity, the oe group showed higher Ki67 staining intensity compared to the oe-NC group. \*\*\*p < 0.001.



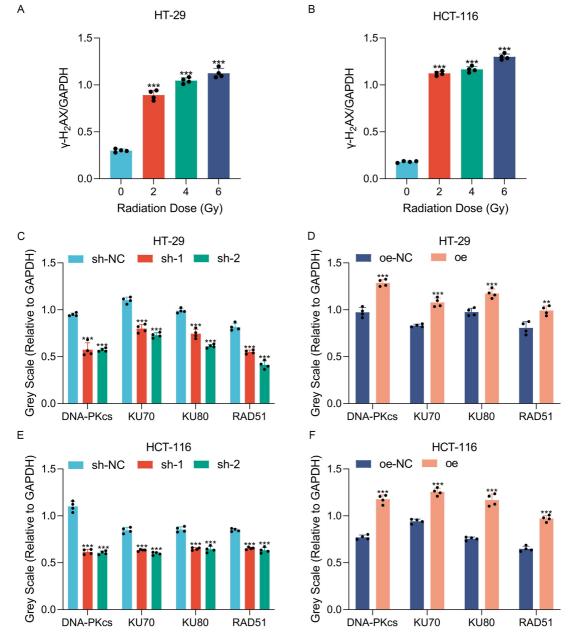
**Supplementary Figure S7:** (A) IHC staining analysis of BANF1 in subcutaneous tumor models utilizing nude mice. (B) Representative HE staining images illustrating BANF1 expression in subcutaneous tumor models.



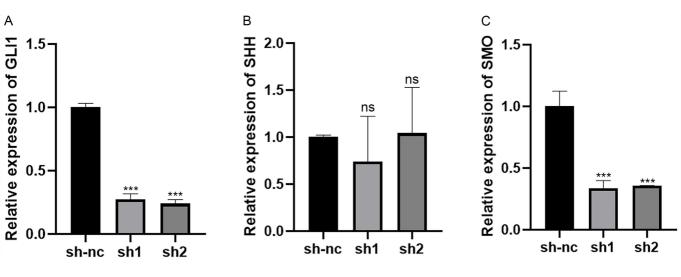
**Supplementary Figure S8:** (A-D) A quantitative assessment of the Western blot results for epithelial-mesenchymal transition (EMT)-related markers in cell lines with BANF1 knockdown and overexpression. \*\*\*p < 0.001.



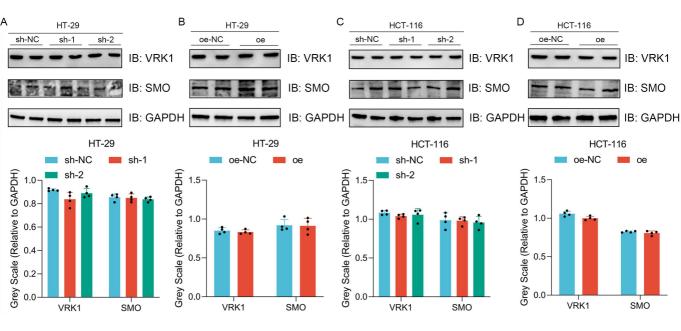
**Supplementary Figure S9:** (A-B) As radiation dose increases,  $\gamma$ -H2AX protein levels rise in HT-29 and HCT-116 cellsAs radiation dose increases,  $\gamma$ -H2AX protein levels rise in HT-29 and HCT-116 cells. (C-E) In HT-29, BANF1 knockdown reduces colony survival at 2 Gy, 4 Gy, and 6 Gy, while overexpression increases survival. (F-H) In HCT116, BANF1 knockdown enhances radiotherapy sensitivity, whereas overexpression decreases it. (I-L) Both cell lines show reduced DNA-PKcs, KU70, KU80, and RAD51 levels in sh-1 and sh-2 groups compared to sh-NC, while overexpression elevates these levels compared to oe-NC. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.



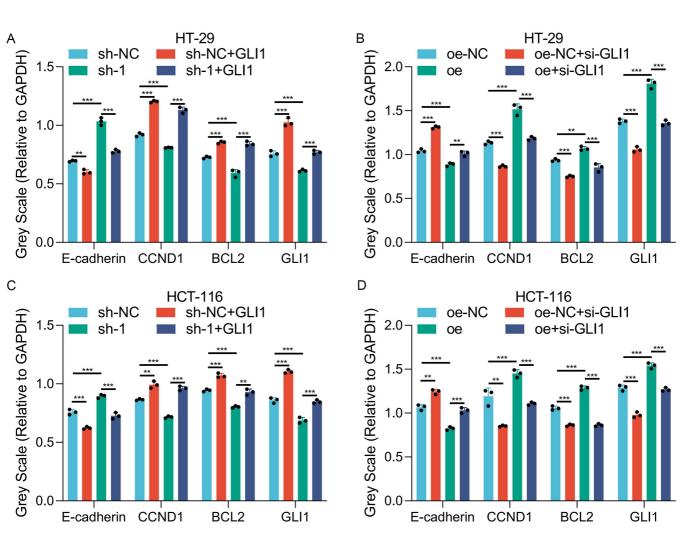
Supplementary Figure S10: The gray scale analysis of  $\gamma$ -H2AX in HT-29 (A) and HCT-116 (B) cell lines following radiation treatment is presented. (C-F) The quantitative analysis of western blotting results pertaining to DNA damage repair-related markers in cell lines with BANF1 knockdown and overexpression were shown. \*\*p < 0.01; \*\*\*p < 0.001.



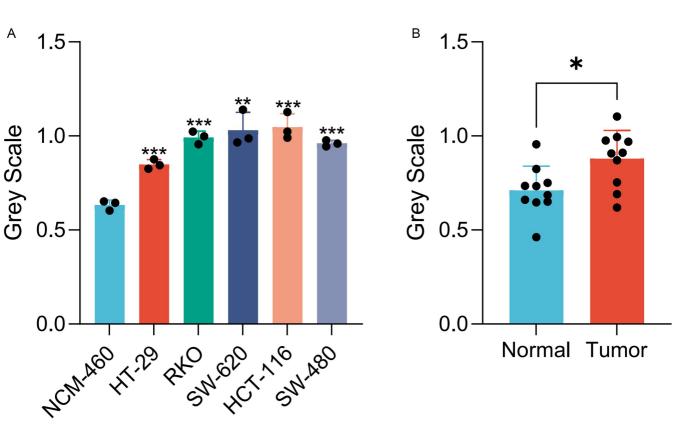
**Supplementary Figure S11:** qRT-PCR analysis was conducted to assess the mRNA expression levels of markers associated with the Hedgehog signaling pathway in both control and BANF1 knockdown HCT-116 cell lines. The markers analyzed included GLI1 (A), SHH (B), and SMO (C). ns: no significance; \*\*\*p < 0.001.



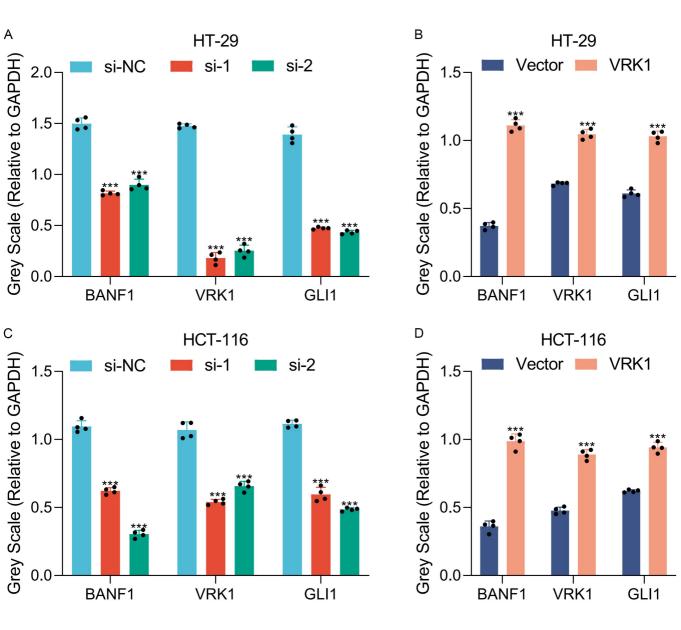
**Supplementary Figure S12:** (A-D) The modulation of BANF1 expression, either through knockdown or overexpression, did not affect the protein levels of VRK1 and SMO.



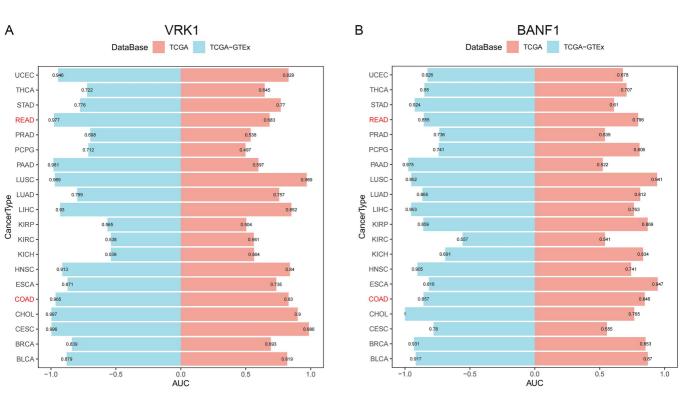
**Supplementary Figure S13:** (A-D) Quantitative analysis of the results obtained from western blotting in the context of rescue experiments. \*\*p < 0.01; \*\*\*p < 0.001.



**Supplementary Figure S14:** Quantitative analysis of Western blot data for VRK1 expression in CRC cell lines (A) and tissue samples (B). p < 0.05; p < 0.01; p < 0.01; p < 0.01.



**Supplementary Figure S15:** (A-D) Quantitative analysis of western blot results for BANF1, VRK1, and GLI1 following VRK1 knockdown and overexpression in HT-29 and HCT-116 cell lines. \*\*\*p < 0.001.



**Supplementary Figure S16:** The AUC values for VRK1 (A) and BANF1 (B) in both normal and tumor tissues were analyzed using the TCGA and GTEx datasets.

Pathway	-log10(pvalue)	NES	Level 3 of KEGG functional Category	(1	Level 2 of KEGG functional Category	Level 1 of KEGG functional Category
ko04110	10	2.34	Cell cycle		Cell growth and death	
ko04114	2.09	1.44	Oocyte meiosis			
ko04810	4.92	-1.75	Regulation of actin cytoskeleton		Cell motility	
ko04520 ko04510	3.07	-1.72 -2.58	Adherens junction			
ko04510	3.59	-2.56	Focal adhesion Gap junction		Cellular community – eukaryotes	Cellular Processes
ko04530	3.2	-1.66	Tight junction			
ko04140	2.65	1.82	Autophagy – animal	- í		
ko04144	2.65	-1.49	Endocytosis		Transport and catabolism	
ko04142	1.72	-1.43	Lysosome			
ko02010	3.13	-1.84	ABC transporters	!	Membrane transport	
ko04020	10	-2.35	Calcium signaling pathway			
ko04012 ko04340	2.29	-1.56	ErbB signaling pathway			
ko04340	4.69	-1.68	Hedgehog signaling pathway MAPK signaling pathway			
ko04330	3.33	-1.86	Notch signaling pathway		Signal transduction	
ko04070	2.69	-1.66	Phosphatidylinositol signaling system			Environmental Information Processing
ko04350	4.96	-2.02	TGF-beta signaling pathway			
ko04310	4.79	-1.85	Wnt signaling pathway			
ko04514	7.66	-2.12	Cell adhesion molecules			
ko04512	10	-2.91	ECM-receptor interaction		Signaling molecules and interaction	
ko04080 ko03050	5.81	-1.77 2.15	Neuroactive ligand-receptor interaction Proteasome			
ko03060	4.96 2.63	1.91	Protein export			
ko03000	3.93	1.99	RNA degradation		Folding, sorting and degradation	
ko04141	1.36	-1.6	Protein processing in endoplasmic reticulum			
ko03410	4.45	2.13	Base excision repair	í í		
ko03030	5.82	2.32	DNA replication			Genetic Information Processing
ko03440	4.61	2.19	Homologous recombination		Replication and repair	Genetic Information Processing
ko03430	2.65	1.91	Mismatch repair			
ko03420	5.73	2.25	Nucleotide excision repair		Townshields	
ko03040 ko00970	5.39	1.93	Spliceosome Aminoacyl-tRNA biosynthesis		Transcription	
ko03010	7.92	2.3	Ribosome		Translation	
ko05200	6.46	-1.74	Pathways in cancer	- i.	Cancer: overview	
ko05221	1.97	-1.55	Acute myeloid leukemia	- î.		
ko05217	5.52	-2.2	Basal cell carcinoma		Cancer: specific types	
ko05213	1.38	-1.45	Endometrial cancer		Cancer: specific types	
ko05218	1.46	-1.44	Melanoma	!		
ko05412 ko05414	7.18	-2.26	Arrhythmogenic right ventricular cardiomyopathy Dilated cardiomyopathy		Cardiovascular disease	Human Diseases
k005414	6.23	-2.11	Hypertrophic cardiomyopathy		Cardiovascular disease	
ko04930	2	-1.58	Type II diabetes mellitus	- i	Endocrine and metabolic disease	
ko05320	3.67	1.95	Autoimmune thyroid disease	- i.	Immune disease	
ko05016	3.46	1.63	Huntington disease	í.	New dependence discourse	
ko05012	4.7	1.9	Parkinson disease		Neurodegenerative disease	1
ko00280	2.33	1.68	Valine, leucine and isoleucine degradation	Į.	Amino acid metabolism	1
ko00650 ko00020	1.78	1.61	Butanoate metabolism			
ko00020	1.48	1.52	Citrate cycle (TCA cycle) Glyoxylate and dicarboxylate metabolism		Carbohydrate metabolism	
ko00030	1.91	1.73	Nitrogen metabolism			
ko00190	7.21	2.17	Oxidative phosphorylation		Energy metabolism	
ko00532	2.98	-1.94	Glycosaminoglycan biosynthesis - chondroitin sulfate / de	lermatar	Glycan biosynthesis and metabolism	Metabolism
ko01040	1.44	1.57	Biosynthesis of unsaturated fatty acids	i	Lipid metabolism	1
ko00670	1.78	1.74	One carbon pool by folate	1	Metabolism of cofactors and vitamins	
ko00480 ko00430	2.31	1.7 -1.88	Glutathione metabolism		Metabolism of other amino acids	1
ko00430	1.96	-1.88	Taurine and hypotaurine metabolism Terpenoid backbone biosynthesis			
ko00240	3.38	1.75	Pyrimidine metabolism		Metabolism of terpenoids and polyketides Nucleotide metabolism	
ko04270	6.04	-2.07	Vascular smooth muscle contraction	- i -	Circulatory system	
ko04360	10	-2.48	Axon guidance	- i	Development and regeneration	
ko04912	2.57	-1.61	GnRH signaling pathway	- î		
ko04910	1.37	-1.33	Insulin signaling pathway		Endocrine system	
ko04916	4.19	-1.84	Melanogenesis	!		
ko04710 ko04960	2.2	-1.85	Circadian rhythm Aldosterone-regulated sodium reabsorption		Environmental adaptation	1
ko04900	2.36	1.65	Antigen processing and presentation		Excretory system	1
ko04623	5.09	2.18	Cytosolic DNA-sensing pathway			Organismal Systems
ko04622	2.31	1.71	RIG-I-like receptor signaling pathway			1
ko04620	1.32	1.34	Toll-like receptor signaling pathway		Immune system	1
ko04610	5.86	-2.15	Complement and coagulation cascades			1
ko04666	1.71	-1.46	Fc gamma R-mediated phagocytosis			1
ko04670 ko04722	2.67	-1.59	Leukocyte transendothelial migration	. !		1
ko04722 ko04740	1.76	4.16	Neurotrophin signaling pathway Olfactory transduction		Nervous system Sensory system	1
	0.0 2.5 5.0 7.5 10.0	-2 0 2 4	,		Genauly System	1
	1.0 10.0	2 2 4				

**Supplementary Figure S17:** GSEA was conducted to assess the biological functions and associated pathways of VRK1.

Supplementary Table S1: The primer sequences for PCR amplification.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
BANF1	TGGCTGAAAGACACTTGTGG	CACTCTCGAAGGCATCCGAA G
GAPDH	GGGAAGGTGAAGGTCGGAGT	GGGGTCATTGATGGCAACA

## Supplementary Table S2: Antibodies used in this study.

Anti-BANF1 antibody (Zhengneng Biological, China), used for immunohistochemistry; Anti-BANF1 antibody (Santa Cruz Biotechnology, USA), used for western blotting; Anti-GAPDH antibody (Santa Cruz Biotechnology, USA), used for western blotting; Rabbit secondary antibody, mouse secondary antibody (Biyuntian, China); Anti-BANF1 antibody (Abcam, Cambridge), for Western blot analysis; Anti-GAPDH antibody (Santa, USA), for Western blot analysis; Anti-Ki67 antibody (ABclonal, China), for immunofluorescence staining; Anti-cleaved-caspase3 antibody (Affinity), for both Western blot and immunofluorescence staining; Anti-CCND1 antibody (Zhengneng Biotech, China), for Western blot analysis; Anti-E-cadherin antibody (Proteintech, China), for Western blot analysis; Anti-N-cadherin antibody (ImmunoWay, USA), for Western blot analysis; Anti-Vimentin antibody (ImmunoWay, USA), for Western blot analysis; Anti-Snail antibody (ImmunoWay, USA), for Western blot analysis; Anti-BCL2 antibody (ImmunoWay, USA), for Western blot analysis; Anti-caspase3 antibody (ABclonal, China), for Western blot analysis; Anti-DNAPKcs antibody (Zhengneng Biotech, China), for Western blot analysis; Anti-KU70 antibody (Zhengneng Biotech, China), for Western blot analysis; Anti-KU80 antibody (Zhengneng Biotech, China), for Western blot analysis; Anti-RAD51 antibody (ImmunoWay, USA), for Western blot analysis; Rabbit secondary antibody, mouse secondary antibody (Biyuntian, China), for Western blot analysis. Anti-BANF1 antibody (rabbit, Abcam, Cambridge), employed for Western blotting and immunofluorescence staining; Anti-VRK1 antibody (rabbit, Immunoway, USA), used for Western blotting; Anti-BANF1 antibody (rabbit, Thermo Fisher Scientific, USA), utilized for immunoprecipitation; Anti-VRK1 antibody (mouse, Santa Cruz Biotechnology, USA), applied in immunofluorescence staining and immunoprecipitation; Mouse IgG Isotype Control (Zhengneng, China); Rabbit IgG Isotype Control (Zhengneng, China).

Supplementary Table S3: The sequences of siRNAs.

si-RNA	sequences
si-GLI1	5'-CUCCACAGGCAUACAGGAU-3'
si-VRK1-1	5'-GCAGUUGGAGAGAUAAUAATT-3'
si-VRK1-2	5'-GCAGCUAAGCUUAAGAAUUTT-3'

Supplementary Table 54. Chinear information of patients with CRC.								
id	age	sex	CEA	Stage	status	time	BANF1	
P8	<65	female	>=5	III	0	1895	low	
P2	<65	female	<5	II	1	1811	low	
P39	<65	female	<5	II	0	2556	high	
P17	<65	male	<5	IV	1	2330 702	low	
P30	<65	female	<5	II	0			
						2610	low	
P41	<65	female	<5	II	0	2433	high	
P48	<65	female	<5	III	1	728	high	
P43	<65	female	>=5	II	0	2181	high	
P40	<65	female	<5	II	0	2487	high	
P67	<65	female	>=5	III	1	1770	high	
Р9	<65	male	<5	III	0	1559	low	
P57	<65	female	>=5	II			high	
P4	<65	male	>=5	II	1 0	356 2040	low	
P16	<65	female	<5	III	1	386	low	
P26	<65	female	<5	III	0	1852	low	
P11	<65	male	<5	III	0	1284	low	
P13	<65	female	<5	III	1	613	low	
P54	<65	female	>=5	Π	0	2050	high	
P7	<65	male	>=5	III	0	2405	low	
P1	<65	male	<5	II	0	2320	low	
P34	<65	male	<5	II	1	2388	high	
P24	<65	male	<5	III	0	1887	low	
P27	<65	female	<5	Ι	0	2143	low	
P23	<65	male	<5	II	0	1833	low	
P62	<65	female	>=5	III	0	2027	high	
P47	<65	male	>=5	III	1	333	high	
P15	<65	male	<5	III	0	1980	low	
P49	<65	male	<5	IV	1	1127	high	
P60	<65	male	<5	III	0	2034	high	
P61	<65	male	<5	III	0	2034	high	
P10	<65	male	>=5	III	0	2402	low	
P53	<65	female	>=5	IV	1	1464	high	
P68	<65	female	>=5	IV	1	1006	high	
P31	<65	female	<5	II	0	2610	low	

**Supplementary Table S4:** Clinical information of patients with CRC.

## Supplementary Table S4: Clinical information of patients with CRC.

P35	<65	female	<5	II	0	2310	high
P44	<65	male	>=5	II	0	2172	high
P58	<65	female	>=5	Π	0	2029	high
P52	<65	female	>=5	IV	1	342	high
P28	<65	female	<5	Ι	0	2046	low
P33	<65	female	<5	II	1	2475	high
P56	<65	male	>=5	II	1	1890	high
P59	<65	male	<5	II	1	2014	high
P6	>=65	male	>=5	II	0	2309	low
P14	>=65	female	>=5	III	1	36	low
P37	>=65	male	<5	II	0	2110	high
P46	>=65	female	>=5	III	1	352	high
P25	>=65	male	<5	III	0	1863	low
P38	>=65	male	<5	II	1	2725	high
P3	>=65	female	<5	II	0	2173	low
P21	>=65	female	>=5	II	0	1734	low
P55	>=65	female	>=5	II	1	288	high
P18	>=65	male	<5	II	0	1910	low
P29	>=65	female	<5	Ι	1	1344	low
P5	>=65	female	>=5	Π	0	2004	low
P42	>=65	female	<5	Π	0	2223	high
P22	>=65	male	<5	II	1	129	low
P12	>=65	male	<5	III	0	1117	low
P32	>=65	male	<5	II	0	2543	low
P51	>=65	male	>=5	IV	1	69	high
P50	>=65	male	<5	IV	1	83	high
P63	>=65	male	>=5	III	1	1141	high
P65	>=65	female	>=5	III	1	630	high
P20	>=65	female	<5	II	0	1897	low
P64	>=65	male	>=5	III	1	83	high
P36	>=65	male	<5	II	1	2257	high
P45	>=65	female	>=5	II	1	1157	high
P66	>=65	male	>=5	III	1	708	high
P19	>=65	female	<5	II	1	2173	low